IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Nisson *et al.*	Confirmation No.:
Appl. No. To be assigned (Continuation of 09/103,577, filed June 24, 1998, allowed)	Art Unit: To be assigned
Filed: (herewith, April 10, 2001)	Examiner: To be assigned
For: Method for Isolating and Recovering Target DNA or RNA Molecules Having a Desired Nucleotide Sequence	Atty. Docket: 0942.4800002/RWE/ALS

Preliminary Amendment and Submission of Substitute Sequence Listing

Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants submit the following Amendment and Remarks. This Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks and arguments. 37 C.F.R. § 1.111 and MPEP 714; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying

this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

Amendments

In the Specification:

Please substitute the paragraph beginning on page 1, line 10, with the following paragraph:

This application claims the benefit under 35 U.S.C. §120 of U.S. Application No. 09/103,577 (allowed), filed on June 24, 1998, which claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 60/050,729, filed on June 25, 1997, both herein incorporated by reference in their entirety.

Please delete pages 42-46 which contain a sequence listing. Please insert the substitute sequence listing provided herewith at the end of the application. Please renumber the pages accordingly.

In the Claims:

Please cancel claim 5 without prejudice or disclaimer.

Please substitute the following claim 1 for the pending claim 1:

Claim 1 (amended). A method for denaturing or separating double-stranded nucleic acid molecules, said method comprising contacting one or more double-stranded nucleic acid molecules with a denaturant selected from the group consisting of one or more amino acid denaturants, imidazole, and one or more amino acid denaturants plus imidazole, thereby forming single-stranded nucleic acid molecules, with the proviso that said denaturant is not selected from the group consisting of asparagine and β-alanine.

Please substitute the following claim 2 for the pending claim 2:

Claim 2 (amended). The method of claim 1, wherein said amino acid denaturants are selected from the group consisting of one or more amino acids, polyamino acids, and combinations thereof; wherein said amino acid denaturants denature or separate double-stranded nucleic acid molecules.

Please substitute the following claim 3 for the pending claim 3:

Claim 3 (amended). The method of claim 41, wherein said polyamino acids comprise two or more amino acids.

Please substitute the following claim 4 for the pending claim 4:

Claim 4 (amended). The method of claim 41, wherein said amino acid denaturants are selected from the group consisting of glycine, D-alanine, L-alanine, DL-alanine, arginine, glutamine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Please substitute the following claim 6 for the pending claim 6:

Claim 6 (amended). The method of claim 1, wherein the concentration of said denaturants ranges from about 1mM to about 500 mM.

Please substitute the following claim 9 for the pending claim 9:

Claim 9 (amended). A method of recovering one or more desired target nucleic acid molecules from a population of nucleic acid molecules comprising:

a) contacting said population with one or more haptenylated probes to permit said probe to hybridize to said desired target molecules thereby forming one or more hybridized molecules; and

b) isolating said desired target nucleic acid molecules from said probes by contacting said hybridized molecules with a denaturant selected from the group consisting of one or more amino acid denaturants, imidazole, and one or more amino acid denaturants plus imidazole.

Please substitute the following claim 19 for the pending claim 19:

Claim 19 (amended). The method of claim 18, further comprising treating said double-stranded nucleic acid molecules to render such molecules single-stranded.

Please substitute the following claim 20 for the pending claim 20:

Claim 20 (amended). The method of claim 19, wherein said treatment comprises contacting said double-stranded nucleic acid molecule with a denaturant selected from the group consisting of one or more amino acid denaturants, imidazole, and one or more amino acid denaturants plus imidazole.

Please substitute the following claim 23 for the pending claim 23:

Claim 23 (amended). The method of claim 9, further comprising (c) synthesizing a nucleic acid molecule complementary to said desired target molecules and forming double-stranded nucleic acid molecules.

Please substitute the following claim 27 for the pending claim 27:

Claim 27 (amended). The method of claim 26, wherein said nucleotide analogs are methylated nucleotides.

Please substitute the following claim 36 for the pending claim 36:

Claim 36 (amended). The method of claim 35, wherein said enriching comprises separating the desired nucleic acid molecules according to size.

Please add the following new claims:

Claim 41. (new) The method of claim 2, wherein said amino acid denaturants are natural or unnatural amino acids.

Claim 42 (new). A method of enriching one or more desired target nucleic acid molecules from a population of nucleic acid molecules comprising:

a) denaturing or separating double-stranded nucleic acid molecules within said population of nucleic acid molecules by contacting one or more double-stranded nucleic acid molecules with a denaturant selected from the group consisting of one or more amino acid denaturants, imidazole, and one or more amino acid denaturants plus imidazole, thereby forming single-stranded nucleic acid molecules;

b) contacting said single-stranded nucleic acid molecules with one or more primers complementary to one or more sequences of the desired target nucleic acid molecules; and

c) amplifying said desired target nucleic acid molecules;

wherein said desired target nucleic acid molecules are enriched.

Claim 43 (new). The method of claim 42, further comprising incubating said single-stranded nucleic acid molecules and said one or more primers with one or more nucleotides and a polypeptide having polymerase activity under conditions to generate one or more synthesized nucleic acid molecules complementary to said desired target molecules, thereby forming double-stranded nucleic acid molecules.

Claim 44 (new). The method of claim 43, wherein said nucleotides confer nuclease resistance to said one or more synthesized nucleic acid molecules.

Claim 45 (new). The method of claim 44, wherein said nucleotides are nucleotide analogs.

Claim 46 (new). The method of claim 45, wherein said nucleotide analogs are methylated nucleotides.

Claim 47 (new). The method of claim 46, wherein said methylated nucleotides are 5-methyldeoxycytosine.

Claim 48 (new). The method of claim 47, further comprising digesting one strand of said double-stranded nucleic acid molecules with one or more nucleases, thereby forming digested molecules.

Claim 49 (new). The method of claim 48, further comprising transforming said digested molecules into one or more host cells.

Claim 50 (new). The method of claim 43, further comprising transforming said double-stranded molecules into one or more host cells.

Claim 51 (new). The method of claim 42, wherein said amino acid denaturants are selected from the group consisting of one or more amino acids, polyamino acids, and combinations thereof; wherein said amino acid denaturants denature or separate double-stranded nucleic acid molecules.

Claim 52 (new). The method of claim 51, wherein said amino acid denaturants are natural or unnatural amino acids.

Claim 53 (new). The method of claim 52, wherein said amino acid denaturants are selected from the group consisting of glycine, D-alanine, L-alanine, DL-alanine, arginine, glutamine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

Claim 54 (new). The method of claim 53, wherein said denaturant is glycine.

Claim 55 (new). The method of claim 52, wherein said amino acid denaturants comprise two or more amino acids.

Claim 56 (new). The method of claim 42, wherein the concentration of said denaturant ranges from about 1mM to about 500 mM.

Claim 57 (new). The method of claim 56, wherein said concentration ranges from about 5 mM to about 50 mM.

Claim 58 (new). The method of claim 57, wherein said concentration is about 10 mM.

Claim 59 (new). The method of claim 42, wherein said one or more primers are degenerate primers.

Claim 60 (new). The method of claim 59, wherein said degenerate primers comprise one or more universal nucleotides.

Claim 61 (new). The method of claim 60, wherein said degenerate primers comprise one or more nucleotides selected from the group consisting of dP and dK.

Claim 62 (new). The method of claim 42, further comprising selecting or enriching for desired target nucleic acid molecules having larger or longer segments from a population of desired target nucleic acid molecules.

Claim 63 (new). The method of claim 62, wherein said enriching comprises separating the desired nucleic acid molecules according to size.

Claim 64 (new). The method of claim 63, wherein said method comprises amplifying the desired nucleic acid molecules prior to size separation.

Claim 65 (new). The method of claim 42, further comprising degradation of one strand of said double-stranded nucleic acid molecules.

Claim 66 (new). The method of claim 65, wherein said degradation comprises the use of Gene II protein and Exonuclease III.

Claim 67 (new). The method of claim 42, wherein said one or more primers comprise a Kozac sequence.

Claim 68 (new). The method of claim 67, wherein said one or more primers are degenerate primers.

Claim 69 (new). The method of claim 42, further comprising separating said one or more double-stranded nucleotide molecules according to size.

Claim 70 (new). The method of claim 42 with the proviso that said denaturant is not selected from the group consisting of asparagine and β-alanine.

Claim 71 (new). The method of claim 42, wherein said population of nucleic acid molecules comprises circular DNA, linear DNA, or a cDNA library.

In the Drawings:

Three (3) sheets of formal drawings corresponding to the informal drawings are submitted herewith. Please substitute the formal drawings for the informal drawings.

Remarks

Sequence Listing:

No new matter has been added. The specification has been amended to direct the entry of this sequence listing after the claims of the above identified application.

In accordance with 37 C.F.R. § 1.821(g), this submission includes no new matter.

In accordance with 37 C.F.R. § 1.821(f), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith in the above application are the same.

Claim Amendments:

Upon entry of the foregoing amendment, claims 1-4 and 6-71 are pending in the application, with claims 1, 9, and 42 being the independent claims. Support for these amendments and newly added claims can be found throughout the specification.

Support for the amendments to claims 1 and 9 can be found on page 16, lines 1-14, specifically on line 11. Support for the amendments to claims 2-4, 6, 19 and 20 can be found, for example, on page 16. Further support for the amendment to claim 20 can be found on page 39, lines 26-31. Support for the amendment to claim 23 can be found on pages 22-23. The amendments to claim 27 and 36 are made to more clearly define the invention.

New claims 41-71 are sought to be added. These changes are believed to introduce no new matter, and their entry is respectfully requested. Support for newly

added claim 41 can be found, for example, on page 16. Support for newly added claim 42 can be found, for example, on page 7, line 29 to page 8 line 31; and on page 24, lines 13-22. Support for newly added claims 43 can be found, for example, on page 7 line 29 to page 8, line 18; and originally filed claim 23. Support for newly added claims 44 and 45 can be found, for example, on page 8, lines 1-4. Support for newly added claims 46 and 47 can be found, for example, on page 25, lines 30-32. Support for newly added claims 48, 65 and 66 can be found, for example, on page 13, lines 23-26 and originally filed claims 21 and 22. Support for newly added claims 49 and 50 can be found, for example, on pages 30-31. Support for newly added claims 51-58 and 70 can be found, for example, on page 16. Support for newly added claim 59 and 68 can be found, for example, on page 18, lines 18-20. Support for newly added claim 60 can be found, for example, from page 17, line 32 to page 18, line 12. Support for newly added claim 61 can be found, for example, on page 18, lines 11-12. Support for newly added claim 62 can be found, for example, on page 8, lines 19-31. Support for newly added claims 63 and 64 can be found, for example, on page 22, lines 6-10. Support for newly added claim 67 can be found, for example, on page 40, lines 15-19. Support for newly added claim 69 can be found, for example, on page 8, lines 19-31; and originally filed claim 36. Support for newly added claim 71 can be found, for example, on page 9 and originally filed claims 10-14.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Robert W. Esmond

Robert W. Esmond
Attorney for Applicants
Registration No. 32,893

Date: April 10, 2001

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Version with markings to show changes made

The paragraph beginning on page 1, line 10 has been replaced by a new paragraph.

The sequence listing on pages 42-46 has been deleted and replaced by a substitute sequence listing at the end of the application.

The three (3) informal drawings have been replaced by three (3) formal drawings.

Claim 5 has been cancelled.

Claim 1 (amended). A method for denaturing or separating double-stranded nucleic acid molecules, said method comprising contacting one or more double-stranded nucleic acid molecules with a denaturant selected from the group consisting of one or more amino acid denaturants, imidazole, and one or more amino acid denaturants plus imidazole, thereby forming [under conditions sufficient to form] single-stranded nucleic acid molecules, with the proviso that said denaturant is not selected from the group consisting of asparagine and β-alanine.

Claim 2 (amended). The method of claim 1, wherein said amino acid denaturants are selected from the group consisting of one or more amino acids, [derivatives, analogs thereof or combinations thereof, and one or more] polyamino acids,

[derivatives, analogs thereof or] and combinations thereof; wherein said amino acid denaturants denature or separate double-stranded nucleic acid molecules.

Claim 3 (amended). The method of claim [2] 41, wherein said polyamino acids comprise two or more amino acids [or derivatives or analogs thereof].

Claim 4 (amended). The method of claim [2] 41, wherein said amino acid denaturants [acids] are selected from the group consisting of glycine, [alanine,] D-alanine, L-alanine, DL-alanine, arginine, [asparagine,] glutamine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine[, and imidazole].

Claim 6 (amended). The method of claim 1, wherein the concentration of said [amino acid] denaturants ranges from about 1mM to about 500 mM.

Claim 9 (amended). A method of recovering one or more desired target nucleic acid molecules from a population of nucleic acid molecules comprising:

a) contacting said population with one or more [hypentylated] haptenylated probes[, under conditions sufficient] to permit said probe to hybridize to said desired target molecules thereby forming one or more hybridized molecules; and

b) isolating said desired target nucleic acid molecules from said probes by contacting said hybridized molecules with a denaturant selected from the group consisting of one or more amino acid denaturants, imidazole, and one or more amino acid denaturants plus imidazole.

Claim 19 (amended). The method of claim 18, further comprising treating said double-stranded nucleic acid molecules [under conditions sufficient] to render such molecules single-stranded.

Claim 20 (amended). The method of claim 19, wherein said treatment comprises contacting said double-stranded nucleic acid molecule with a denaturant selected from the group consisting of one or more amino acid denaturants, imidazole, and one or more amino acid denaturants plus imidazole.

Claim 23 (amended). The method of claim 9, further comprising (c) [incubating said isolated desired target nucleic acid molecules under conditions sufficient to synthesize] synthesizing a nucleic acid molecule complementary to said desired target molecules[, thereby] and forming double-stranded nucleic acid molecules.

Claim 27 (amended). The method of claim 26, wherein said nucleotide analogs are [a] methylated nucleotides.

Claim 36 (amended). The method of claim 35, wherein said enriching [enrichment] comprises separating the desired nucleic acid molecules according to size.

Claims 41-71 are newly added.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: NISSON *et al.*	Confirmation No.
	Art Unit: To be Assigned
Appl. No.: To be assigned (Continuation of 09/103,577, filed June 24, 1998, allowed)	Examiner: To be Assigned
Filed: April 10, 2001	Atty. Docket: 0942.4800002/RWE/ALS
For: **Method for Isolating and Recovering Target DNA or RNA Molecules Having a Desired Nucleotide Sequence**	

Amendment and Submission of Substitute Sequence Listing Under 37 C.F.R. § 1.825(a)

Commissioner for Patents
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. § 1.825(a), Applicants submit substitute sheets to amend the paper copy of the Sequence Listing.

In the Specification:

Please cancel the existing Sequence Listing for the above-identified application, replace it with the substitute Sequence Listing appended hereto, and insert the same at the end of the application.

Remarks

Applicants' undersigned representative hereby states that the change made in the sequence listing does not include new matter. Support for this amendment is found, *inter*

alia, on pages 37, 39 and 40. Applicants' undersigned attorney has amended the specification only to direct the entry of this corrected Sequence Listing at the end of the application.

In accordance with 37 C.F.R. § 1.825(b), the paper copy of the Sequence Listing and the computer readable copy of the Sequence Listing submitted herewith are the same.

It is respectfully believed this application is now in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

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Attorney for Applicants
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Date: April 10, 2001

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: P. Nisson *et al.*	Confirmation No.
Appl. No.: To be assigned (Continuation of 09/103,577, filed June 24, 1998, allowed)	Art Unit: To be assigned
Filed: April 10, 2001	Examiner: To be assigned
For: **Method for Isolating and Recovering Target DNA or RNA Molecules Having a Desired Nucleotide Sequence**	Atty. Docket: 0942.4800002/RWE/ALS

Letter to PTO Draftsman: Submission of Formal Drawings

Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith are three sheets of formal drawings with Figure(s) 1-3, corresponding to the informal drawing(s) submitted with the above-captioned application. Identification of the drawing(s) is provided in accordance with 37 C.F.R. § 1.84(c). Acknowledgment of the receipt, approval, and entry of these formal drawing(s) into this application is respectfully requested.

It is not believed that an extension of time is required, other than any already provided herewith. However, if an extension of time is needed to prevent abandonment of the application, then such extension of time is hereby petitioned. The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036. A duplicate copy of this Letter is enclosed.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Robert W. Esmond

Robert W. Esmond
Attorney for Applicants
Registration No. 32,893

Date: April 10, 2001

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